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EDITORIAL COMMENT

Protein Therapeutics for Cardiovascular Disease

It Is All About Delivery*

Tomoko Ichiki, MD, PhD, John C. Burnett, Jr, MD

Rochester, Minnesota

During the last 2 decades, there have been seminal advances in innovative therapeutics for human disease, which have markedly reduced the burden of diseases in all clinical areas, resulting in improved longevity and quality of life. This impressive progress has been built on the creation of small molecules taking advantage of a new level of expertise in biological and medicinal chemistry. However, during the last few years, we have witnessed a return to the use of proteins and small peptides as innovative therapeutics, in part, due to breakthroughs made by genomic medicine and importantly by advances in protein delivery platforms. This revival of interest in proteins and peptides has also been driven by the decreasing approval of new small molecule drugs.

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What is clear is that proteins and peptides as therapeutics hold great promise. Proteins and peptides go beyond small molecules in terms of both specificity and safety as ligands of human receptors and activators of specific molecular pathways, which as targets may achieve therapeutic goals (1). In cardiovascular diseases, protein therapeutics is now an exploding field. Today we are witnessing the emergence of a diverse field of clinical and pre-clinical investigations using proteins and peptides for a wide spectrum of cardiovascular disease syndromes. For chronic ischemic disease syndromes such as coronary artery disease and peripheral artery disease, the ability to locally infect the heart or peripheral limb with genes, which produce the peptide vascular endothelial growth factor, has been under investigation for over a decade. Indeed, recent breakthroughs have been reported to enhance and simplify delivering with integrating the vascular endothelial growth factor gene with a responsive hypoxic element with nanoparticles to facilitate gene uptake and regulation (2). The gene therapy approach represents a strategy for cardiovascular protein therapeutics to enhance the endogenous production of specific proteins.

In contrast to gene therapy, actual protein therapy and delivery use well-defined and precisely structured proteins or peptides, with previously defined therapeutic doses of an individual protein or peptide for a specific disease state, and with well-characterized biological actions. The use of peptides is expanding in the broad area of heart failure and beyond using natriuretic peptides and particulate guanylyl cyclase receptor agonists as chronic therapeutics. As these guanylyl cyclase receptors possess antihypertrophic, antifibrotic, proangiogenic, endothelial regenerating, aldosterone suppressing, and renal preserving or enhancing properties, these novel guanylyl cyclase activating peptides are in trials for the inhibition of post–acute myocardial infarction (AMI) remodeling, as well as delaying ventricular remodeling in mild systolic heart failure. The latter is delivered by chronic subcutaneous administration (3,4).

In this issue of the *Journal,* Kawata et al. (5), advance the field of protein therapeutics for AMI in elegant pre-clinical research. Their strategy is to go beyond an interventional approach and take advantage of thrombolytics and a novel delivery system to achieve rapid coronary recanalization. It is important to note that the area of thrombolysis represents perhaps cardiology's longest use of protein therapeutics. The changing practice of cardiology and the pivotal improvement in AMI treatment began with the discovery of the protein streptokinase by William Smith Tillett in 1933, and the continuing landmark clinical development by his student Sol Sherry, which laid the foundation for its use as a thrombolytic agent in the treatment of AMI (6,7).

Currently, reperfusion therapy for AMI is recommended within 12 h from the onset of AMI to re-establish myocardial blood flow from the culprit lesion to reduce myocardial necrosis and remodeling, which would improve mortality and morbidity in AMI patients. From several clinical trials, percutaneous coronary intervention (PCI) demonstrated favorable results as compared with thrombolytic therapy; however, a certain number of patients still required thrombolysis in individual settings, such as patient characteristics, time from onset, or accessing a hospital where there are emergency PCI capabilities in place (8). The main issues of thrombolytic therapy that remain are a less successful (= reperfusion) rate and higher risk of serious hemorrhage than PCI.

In this seminal study by Kawata et al. (5), who elegantly focused on thrombolytic therapy to improve those issues, the investigators report the development of a "stealth" technology in which a thrombolytic protein (tissue type

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From the Cardiorenal Research Laboratory, Division of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota. This work was supported by grants from the National Institute of Health (RO1 HL36634, PO1 HL76611, and RO1 HL83231) awarded to Dr. Burnett, Jr., and from the American Heart Association with a Scientist Development Grant awarded to Dr. Ichiki (12SDG11460017) and the Mayo Foundation. Dr. Burnett is the Chair of the Scientific Advisory Board of Nile Therapeutics and a Scientific Advisory to Anexon to which Mayo Clinic has licensed the peptide Cenderitide and ANX042, respectively.

plasminogen activator [tPA]) is encapsulated into gelatin, a large protein that recognizes von Willebrand factor, which is expressed on the surface of platelets. This therapeutic protein platform together with zinc ions suppresses tPA activity in vitro and can be activated in vivo by ultrasound, which therefore represents the "stealth" delivery platform permitting the protein complex to circulate with little activity and which then can be activated ultrasonically near an active thrombus (9). This highly novel pre-clinical approach advanced by the investigators was comprehensive with testing of the concept of a gelatin, tPA, and zinc complex in vitro and then taking this protein delivery system to a mouse model of thrombosis to establish potential feasibility. Most importantly, they then translated their findings to a swine model of AMI.

The key findings were that these gelatin nanoparticles with tPA bind to von Willebrand factor ex vivo, and in a mouse model of thrombus these nanoparticles accumulate at the site of thrombosis. In the swine model of AMI, circulating levels of tPA were modest after injection of the gelatin/tPA nanoparticles, thus protecting tissues remote from coronary thrombosis from high active tPA concentrations. Importantly, transthoracic ultrasound locally and completely activated these tPA-carrying nanoparticles in the model of AMI. Indeed, this innovative protein therapeutic strategy recanalized 9 of 10 pigs in 30 min. These findings may translate into an improved AMI therapeutic strategy with thrombolysis because of the novel tPA nanoparticles, which allow targeting only the fresh thrombosis and do so with lower doses, which may decrease a risk of serious bleeding complications.

These studies are of high clinical importance, and as stated by the investigators, this innovative drug delivery system "may provide novel reperfusion therapy in AMI patients which can be theoretically started in an ambulance, and is also applicable in the treatment of stroke, pulmonary thromboembolism, peripheral arterial thrombosis and leg vein thrombosis." The investigators are to be congratulated for their pioneering work and are anxious to expand the study into other thrombotic diseases as well in the future.

It is worth making some additional comments that build on the investigators' statement, which implies the use of early intervention with this novel reperfusion technology. First, cardiology and the healthcare delivery system have made substantial progress in door-to-balloon time for AMI. Krumholz et al. (10), writing in 2011, reported an analysis of all patients reported by hospitals to the Centers for Medicare and Medicaid Services for inclusion in the time-to-PCI inpatient measure from 2005 to 2010. Their report clearly documented impressive progress in the United States in reducing door-to-balloon time. To complement this recent report is the important study by Shiomi et al. (11) from Japan. These investigators reported key findings from the CREDO-Kyoto (Coronary Revascularization Demonstrating Outcome Study in Kyoto) study on onset-to-balloon versus door-to-balloon times and outcomes. Importantly, short onset-to-balloon time was associated with better 3-year outcomes in ST-segment elevation AMI patients having PCI, whereas the benefit of short door-to-balloon time was limited to patients who presented early. These studies shift our attention to this critical period prior to hospital arrival. Perhaps the "next frontier" of technologies advanced by Kawata et al. (5) offers a view to the future in which a specialized mobile emergency chest pain unit could emerge that travels to the patient. Such a mobile unit with electrocardiogram telemedicine and a point of care troponin measurement together with clinical judgment establish the ST-segment elevation AMI diagnosis. An intravenous injection of tPA nanoparticles follows and they are activated by transthoracic ultrasound at the site of coronary thrombosis to initiate immediate reperfusion to be finalized as required in the catheterization laboratory. Clearly, these thoughts represent an idealized healthcare delivery system, but such visions of future AMI therapy are becoming clearer and more feasible.

What are the next steps based on the current study? We believe that continued work in the swine model of AMI might help refine timing and long-term outcomes. Indeed one could also combine the swine AMI model with a swine atherosclerosis model to better mimic human coronary artery disease. Would there be benefit too of chronic therapy following recanalization in the pig? These are all important questions, but it is hoped that the investigators will now begin to lay the foundation for proof-of-concept testing in human studies. Indeed, the technology is exciting and its potential in human AMI is high for success, which now will require clinical trials to confirm safety and efficacy.

In summary, the field of cardiovascular protein therapeutics is undergoing an exciting rejuvenation due to advances in protein biochemistry and novel delivery systems. No doubt, we are at only the beginning of a new era in this therapeutic arena, which should enhance our efforts to reduce the burden of cardiovascular disease.

Reprint requests and correspondence: Dr. Tomoko Ichiki, Cardiorenal Research Laboratory, Mayo Clinic, 200 1st Street SW, Rochester, Minnesota 55905. E-mail: Ichiki.tomoko@mayo.edu.

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